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# EFFECT OF EXTRACTS OF *MURRAYA KOENIGII* SPRENG. AND *MORUS ALBA* LINN. ON THE AGE OF ATTAINMENT OF PUBERTY AND OVARIAN FOLLICULOGENESIS IN RATS

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**ABSTRACT:** An experiment was conducted to evaluate the effects of methanolic extracts of *Murraya koenigii* Spreng. (Curry leaf) and *Morus alba* Linn. (Mulberry leaf) on the age of attainment of puberty, relative ovary and uterus weight and the number of ovarian surface follicles in female *Wistar albino* rats. The rats were reared from 20 to 70 days of age in six groups consisting of eight rats in each group. Group I and II were orally administered with 0.5 ml distilled water and 0.5 ml 10% DMSO, respectively. Group III, IV, V and VI were orally administered with methanolic extracts of *Murraya koenigii* at 500 mg/kg b.w. and 1000 mg/kg b.w. and methanolic extracts of *Morus alba* at 250 mg/kg b.w. and 500 mg/kg b.w., respectively. The significant advancement in the mean age of attainment of puberty was observed along with increase in number of surface follicles on both the ovaries in Group III, IV and VI. Whereas, the relative ovary weight was non significant ( $P>0.05$ ) in all the treated groups, the relative uterus weight was significant ( $P<0.05$ ) in Group IV and Group VI. These observations were attributed to the effects of phytoestrogens present in the methanolic extracts of *Murraya koenigii* Spreng. and *Morus alba* Linn.

**KEYWORDS:** Phytoestrogens, *Murraya koenigii*, *Morus alba*, Puberty, Follicles

received on 13-04-2010

modified on 22-07-2010

accepted on 10-10-2010

available online 15-11-2010

www.jbclinpharm.com

## INTRODUCTION

Certain plants, their extractions and preparations are used in fertility regulation in traditional medicine. *Morus alba* leaves contain isoflavonoids and lignans and *Murraya koenigii* leaves contains genistein, which are groups of hormone like diphenolic phytoestrogens. These phytoestrogens are group of plant derived compounds that structurally and functionally mimic the actions of mammalian estrogen. More specifically, phytoestrogens mimic the action of  $17\beta$ -estradiol and bind weakly to estrogen receptor and induce production of sex hormone binding globulin (SHBG) in the liver and in this way influence sexual hormone metabolism.

Natural or synthetic estrogens have serious consequences on the reproductive cycle in hu-

mans and animals. Precocious vaginal opening, which is an indicator of attainment of puberty could occur in response to actions of estrogens or estrogen-like substances [1]. Phytoestrogens modulate steroidogenesis by increasing ovulation rate and decreasing ovarian follicle atresia [2] and also due to preponed FSH surge by plant's active principles and thereby leading to enhanced follicle population [3]. Feeding of curry leaves to cattle increase fertility rate and it is considered as indigenous technical knowledge [4]. Hence, to evaluate the effects of phytoestrogens that may be possibly present in *Murraya koenigii* Spreng. (Curry leaf) and *Morus alba* Linn. (Mulberry leaf), the present study was undertaken with special reference to advancement in the age of attainment of puberty and development of number of ovarian surface follicles in female rats that indicate folliculogenesis.

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## MATERIALS AND METHODS

### Animals

A total of forty eight Wistar albino female rats procured from small animal house, Department of Livestock Production and Management, Veterinary College, Hebbal, Bangalore were reared from 20 to 70 days of age in six groups (Group I, II, III, IV, V and VI) consisting of eight rats in each group. Permission was obtained from Institutional Animal Ethics Committee with No. 15/LPM/IAEC/2008, dated: 03.01.2009, to conduct the experiment. Each rat was indentified individually in a group with the markings of picric acid on the hairs at different sites of the body. They were maintained in polypropylene rat cages under standard laboratory hygienic conditions, providing balanced laboratory animal feed and water ad libitum.

### Plant material

Fresh green leaves of *Murraya koenigii* Spreng. were obtained from the plants grown in Veterinary College Campus, Hebbal, Bangalore and the fresh green leaves of *Morus alba* Linn. were obtained from the fields maintained by Department of Sericulture at Main Research Station, University of Agricultural Sciences, Hebbal Campus, Bangalore. Both the plants were identified and authenticated by Prof. T. K. Narayana Swamy, Department of Sericulture, University of Agricultural Sciences, GKVK Campus, Bangalore. The leaves were shade dried at room temperature and finely pulverized.

### Preparation of extract

The powder prepared from shade dried leaves was extracted directly with methanol using Soxhlet extractor as per the procedure standardized [5]. Methanol was used in the present study with the opinion that the majority of phytochemicals or phytosteroids will get dissolved in organic solvents like methanol [6]. The extracts were stored in desiccators until further use.

### Phytochemical studies

To find out whether the plant extracts contain phytoestrogens, the qualitative phytochemical tests were performed as per the standard procedures mentioned in Indian Pharmacopoeia (1994) and British Pharmacopoeia (1991).

### Animal treatment

Group I (negative control) and Group II (positive control) were administered with 0.5 ml distilled water and 0.5 ml 10% DMSO, respectively, as placebo by oral gavaging technique once daily. The methanolic extracts of *Murraya koenigii* were administered at 500 mg/kg b.w. (Group III) and 1000 mg/kg b.w. (Group IV). The methanolic extract of *Morus alba* was administered at 250 mg/kg b.w. and 500 mg/kg b.w. to the Group V and VI, respectively. The required dose of the plant extract was prepared by dissolving the dried extracted material taken from the desiccated storage in known quantity of 10% DMSO such that finally 0.5 ml of the sample was orally administered by gavaging, once a day.

Throughout the experimental period for each group of female rats one mature male rat was reared at close vicinity to elicit the Whitten effect [7], to uniformly advance the age of attainment of puberty and to synchronize the estrous cycle after attainment of puberty.

### Vaginal opening

Treatment started at the age of twenty days and the opening of vagina, an indicator of attainment of puberty in rats was examined daily at 17.00 hrs from day 35 and onwards by doing canalization through vagina, which normally imperforate before puberty and perforate after attainment of puberty [8].

### Relative organ weights

The rats were sacrificed at the age of 70 days. After sacrificing, they were dissected out and the left and right ovaries were collected in petridish containing 0.9% normal saline. The ovaries were trimmed and blotted with filter paper and weight of the ovaries was recorded with the help of electronic digital balance. Similarly, the weight of the uterus was also recorded. Relative organ weights were calculated by dividing the organ weight (g) by body weight (g) and multiplying by 100 [9].

### Number of Ovarian surface follicles

Total number of surface follicles on both the ovaries were counted with the help of stereozoom microscope to serve as the indicator of folliculogenesis.

### Statistical analysis

The results were expressed as mean  $\pm$  SE. The statistical analysis of the variance between control and the experimental values was carried out using one-way analysis of variance with Dunnett's post test using computerized GraphPad Prism trial Version 5.00 [10].

## RESULTS

### Phytochemical studies

The qualitative phytochemicals tests indicated that the plant extracts contain phytoestrogens or flavonoids.

### Vaginal opening

There was significant ( $P < 0.05$ ) advancement in the age of attainment of puberty in rats, as evidenced by opening of vagina during canalization that was indicated by perforation in different groups, that were administered with the methanolic extracts of *Murraya koenigii* at 500 mg/kg b.w. (Group III), 1000 mg/kg b.w. (Group IV) and *Morus alba* at

500 mg/kg b.w. (Group VI). However, there was non significant ( $P > 0.05$ ) advancement in the age of attainment of puberty in Group V, which were administered with *Morus alba* at 250 mg/kg b.w. The results are compiled in Table 1.

### Relative Organ weights

The mean relative weight of ovaries did not show significant ( $P > 0.05$ ) difference between the groups. Whereas, the relative uterus weight was significant ( $P < 0.05$ ) in Group IV and Group VI, that received *Murraya koenigii* at 1000 mg/kg b.w. and *Morus alba* at 500 mg/kg b.w., respectively. This has indicated that the level of *Murraya koenigii* at 500 mg/kg b.w. and *Morus alba* at 250 mg/kg b.w. was not enough to produce significant effect on uterine weight. The results are compiled in Table 1.

### Ovarian surface follicles

The mean  $\pm$  SE values of number of ovarian follicles were significant in Group III, Group IV and Group VI, that received *Murraya koenigii* at 500 mg/kg b.w., 1000 mg/kg b.w. and *Morus alba* at

**Table 1: Effects of methanolic extracts of *murraya koenigii* spreng. and *morus alba* linn. on reproductive parameters in wistar albino rats**

Treatment	Age of attainment of puberty (days)	Relative weight of Ovary	Relative weight of the Uterus	Number of surface ovarian follicles
Negative control (Distilled water 0.5 ml p.o.)	46.00 $\pm$ 0.46	0.04 $\pm$ 0.002	0.17 $\pm$ 0.01	12.13 $\pm$ 0.77
Positive control (10% DMSO 0.5 ml p.o.)	45.75 $\pm$ 0.49	0.04 $\pm$ 0.003	0.18 $\pm$ 0.01	12.75 $\pm$ 0.65
<i>Murraya koenigii</i> Spreng. extract @ 500 mg/kg b.w. in 0.5 ml of 10% DMSO, p.o.	44.25 $\pm$ 0.37*	0.05 $\pm$ 0.003	0.19 $\pm$ 0.02	15.88 $\pm$ .067**
<i>Murraya koenigii</i> Spreng. extract @ 1000 mg/kg b.w. in 0.5 ml of 10% DMSO, p.o.	44.00 $\pm$ 0.19*	0.05 $\pm$ 0.004	0.27 $\pm$ 0.05*	17.25 $\pm$ 0.70 ***
<i>Morus alba</i> Linn. extract @ 250 mg/kg b.w. in 0.5 ml of 10% DMSO, p.o.	44.50 $\pm$ 0.42	0.05 $\pm$ 0.002	0.21 $\pm$ 0.02	14.00 $\pm$ 0.68
<i>Morus alba</i> Linn. extract @ 500 mg/kg b.w. dissolved in 0.5 ml of 10% DMSO, p.o.	44.13 $\pm$ 0.58*	0.05 $\pm$ 0.005	0.26 $\pm$ 0.01*	16.75 $\pm$ 0.70***

Mean  $\pm$  SE; \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$  compared to control.

500 mg/kg b.w., respectively. Among the positive treatment groups, level of *Morus alba* at 250 mg/kg b.w. was not enough to produce significant effect with respect to folliculogenesis. The results are included in Table 1.

## DISCUSSION

There was advancement in the age of attainment of puberty as evidenced by opening of vagina during canalization that was indicated by perforation in the treatment groups administered with the methanolic extracts of *Murraya koenigii* at 500 mg/kg b.w. (Group III), 1000 mg/kg b.w. (Group IV) and *Morus alba* at 500 mg/kg b.w. (Group VI). However, there was non significant ( $P > 0.05$ ) advancement in the age of attainment of puberty in Group V, which were administered with *Morus alba* at 250 mg/kg b.w.

The significant ( $P < 0.05$ ) advancement in the age of attainment of puberty in rats, in the present study, could be attributed to phytoestrogens present in *Murraya koenigii* and *Morus alba* that exerted positive estrogenic feedback effects on the central nervous system that induced puberty which may be due to stimulation of steroidogenic activity of ovary under the hypothalamic effect. This was in accordance with the observations made by earlier workers [11, 12, 13, 14]. Feeding of phytoestrogens advanced the age of vaginal opening [15] and frank estrogenic compound induced cornification and opening of vagina in immature rats [16].

The relative ovary weights between the groups was non significant ( $P > 0.05$ ). The similar findings were reported earlier [3, 17, 18]. However, increased weight of ovary was noticed in the rats administered with aqueous and ethanolic extracts of *Carum carvi* and *Curcuma longa* which was attributed to the activity of high serum estradiol levels [16].

The significant ( $P < 0.05$ ) uterus weight at higher doses of plant extracts in the present study were in conformity with the conclusions of earlier workers [3, 14, 19, 20, 21] who opined that the feeding of phytoestrogens containing feed increased uterus weight.

The stereo zoom microscopic examination of ovaries revealed that the population of surface follicles on both the ovaries increased significantly in Group III, IV and VI. This enhanced follicle population was attributed to phytoestrogens present in *Murraya koenigii* and *Morus alba* that potentially increased growth and development of follicles and

decreased ovarian follicular atresia. This reduction of atresia might be due to FSH surge caused by phytoestrogens. The results of the present study were in conformity with the findings of previous researchers [2, 3, 22]. The aqueous and ethanolic extracts of *Carum carvi* and *Curcuma longa* exhibited antifertility activity [16].

With the concluding remarks, that the *Murraya koenigii* Spreng. and *Morus alba* Linn. are the potential plants that can cause the significant advancement in the age of attainment of puberty, with a positive role in follicular development, folliculogenesis, and desired effects on development of genital organs in females, further research is proposed that pave the way for extensive use of *Murraya koenigii* Spreng. and *Morus alba* Linn. to derive the beneficial effects such as antifertility activity from these plants that can overcome the reproductive disorders in various domestic animals.

## ACKNOWLEDGEMENT

The staff of the Department of Animal Nutrition, Veterinary College, Hebbal, Bangalore are thankfully acknowledged for providing the facilities in preparing the extracts of the plants.

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